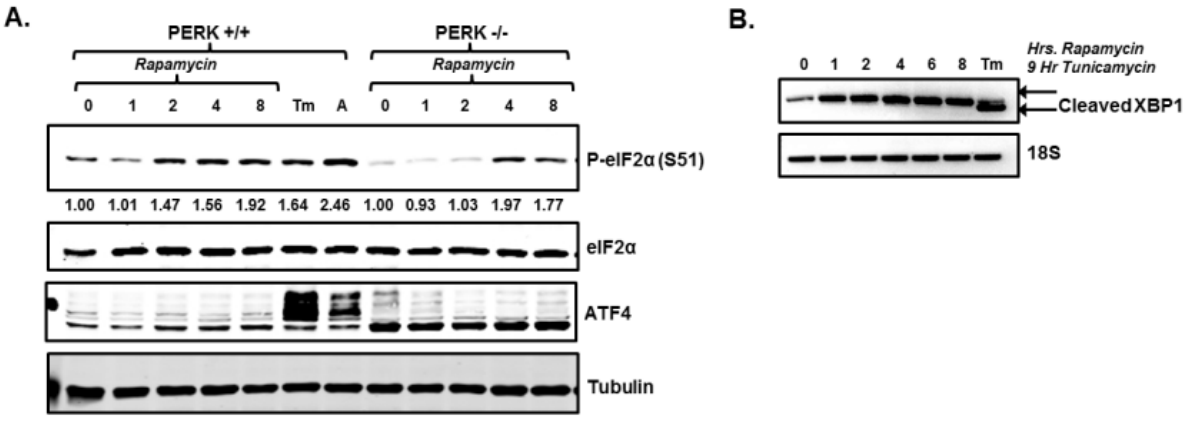
Supplemental Fig 1. mTORC1 inhibition does not lead to phosphorylation of eIF2α through the unfolded protein response A. Wild-type and PERK knockout MEFs were treated with 300nM rapamycin for the indicated times, 2.5μg/mL tunicamycin for 24 hours, or amino acid deprivation for 12 hours. Cell lysates were then immunoblotted for GFP. A representative blot and average quantitation (N=2 biological replicates) are displayed. B. U2OS cells were treated with 300nM rapamycin for the indicated times (hours) or 2.5μg/mL tunicamycin for 9 hours and XBP1 cleavage was determined by PCR. A representative gel from N=2 biological replicates is shown.

Supplemental Fig 2. eIF2α phosphorylation in response to severe amino acid deprivation does not depend on TSC2 status. TSC2 wild-type and TSC2 deficient cells were treated with leucine deprivation for the hours indicated, and eIF2α phosphorylation was assessed. A representative blot from N=2 independent replicates is displayed.

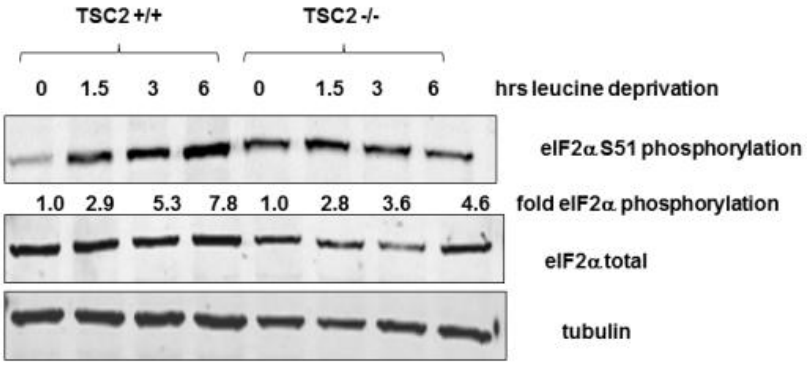
Supplemental Fig 3. PP6C and IGBP1 overexpression regulate rapamycin-induced eIF2α phosphorylation A. U2OS cells stably expressing either a control or PP6C expression retrovirus were deprived of amino acids for 6 hours, treated with 2.5μg/mL tunicamycin for 6 hours, or treated with 300nM rapamycin for the indicated times (hours) and eIF2α phosphorylation was assessed. A representative immunoblot from N=2 biological replicates is shown. B. Control and PP2A depleted cells were treated with rapamycin or tunricamycin (Tm) and eIF2α phosphorylation and p62 expression were assessed. A representative blot and average quantitation (N=2 biological replicates) are displayed. C. U2OS cells stably expressing either a control or IGBP1 expression retrovirus were deprived of amino acids (AA) or treated with 2.5□g/mL tunicamycin (Tm), or 300nM rapamycin (rap) for the indicated times (hours). Protein lysates were then immunoblotted for PP6C, phosphorylated eIF2□ and other noted proteins. A representative immunoblot from N=2 biological replicates is shown.

Supplemental Fig 4. PP6C binding to regulatory subunits affects PP6C stability. A. 293T cells were co-transfected with vector expressing a Myc-tagged PP6C mutant and a vector expressing Flag-PP6R1, Flag-PP6R2, or Flag-PP6R3. Lysates were immunoprecipitated with sepharose beads conjugated to an anti-Flag antibody. Samples were then immunoblotted for the PP6Rs (Flag), and PP6C. A representative immunoblot from N=2 biological replicates is shown. B. Lysates from cells stably expressing a Myc-tagged PP6C mutant or were immunoblotted for PP6C**.** A representative immunoblot from N=2 biological replicates is shown. C. U2OS cells stably expressing a Myc-tagged PP6C expression retrovirus were treated with 100μg/mL cycloheximide for the times indicated. Cell lysates were then immunoblotted for PP6C. A representative immunoblot from N=2 biological replicates is shown.

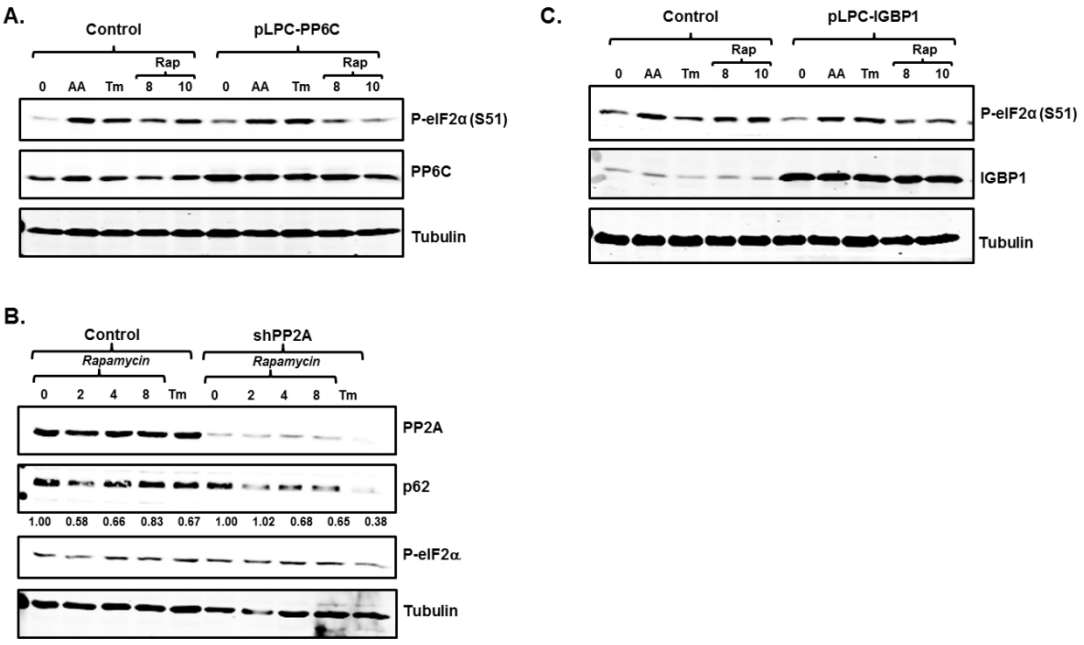
Supplemental Fig 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Fig4